Effect of Hydrogel Porosity on Marrow Stromal Cell Phenotypic Expression

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Introduction: The pore architecture of scaffolds is a significant factor in bone cell function. In this work, the porosity and interconnectivity of microporous oligo-(polyethylene glycol) fumarate (OPF) hydrogels were assessed using scanning electron microscopy (SEM) and micro-magnetic resonance imaging (micro-MRI). We also investigated the effect that morphological features of the OPF hydrogels had on marrow stromal cell adhesion and expression of phenotypic function.

Materials and Methods: OPF was synthesized using polyethylene glycol (PEG) with an initial molecular weight of 10kDa according to a published method.[1]

***Hydrogel fabrication*:** Hydrogels were made by dissolving OPF macromer to a final concentration of 33% (w/w) in deionized water containing 0.05% (w/w) of a photoinitiator (Irgacure 2959, Ciba-Specialty Chemicals) and 0.33% (w/w) N-vinyl pyrrolidinone (NVP). In order to obtain hydrogels with 75%, 80% and 85% porosity, 1 ml of macromer solution was mixed with 3, 4 and 5.7g of sodium chloride particles (100 to 500 μm diameter), respectively and polymerized using 365 nm UV light at an intensity of ~8mW/cm^2 (Blak-Ray) for 10 min.

SEM: Porous hydrogels were dried in a critical point drying apparatus, and fractured using liquid nitrogen. Cross sections of the samples were examined via SEM (S-4700, Hitachi Instruments Inc., Tokyo, Japan).

Micro-MRI: The scaffolds were imaged with a 7 Tesla MRI (Bruker Pharmascan; Bruker Inc, USA). The imaged scaffolds were analyzed using a commercially available image analysis program (Analyze™).

Marrow stromal cell (MSC) isolation, culture and characterization: MSCs were isolated from the femurs and tibiae of male Sprague Dawely rats according to a previously described method.[2] Prior to cell seeding, samples were disinfected with 70% ethanol for 30 min. The ethanol was then aspirated, and the samples were soaked in sterile PBS for 1 h with three changes, followed by two additional changes of media and incubation over night. 25 μl of the cell suspension containing 225,000 cells was seeded onto the top of the hydrogel foams in 24 well plates and incubated for 3 h to allow the cells to attach. 1 ml of osteogenic media was added to each well, and the medium was changed every 2-3 days. At days 1, 4, and 7, samples were washed with PBS three times and frozen in one ml dH_2O at -80°C. Samples underwent two freeze/thaw cycles with sonication on ice for 30 min after each cycle prior to analysis. Cell numbers were determined by the PicoGreen DNA kit (Molecular probes, Eugene, OR) according to the manufacturer's instructions.

Alkaline phosphatase activity was measured using a commercially available kit, according to the manufacturer's instructions (Sigma Chemical).

Results: Figure 1a shows an SEM image of a porous hydrogel with 75% porogen fraction. This picture reveals that the pores are highly interconnected. Figures 1b and c show a representative micro-MR cross-section and the corresponding pore-solid delineation of this scaffold in its swollen state. MR image analysis showed that the pores were highly interconnected and that the porosity computed from the images correlated well with the experimental porogen concentration.

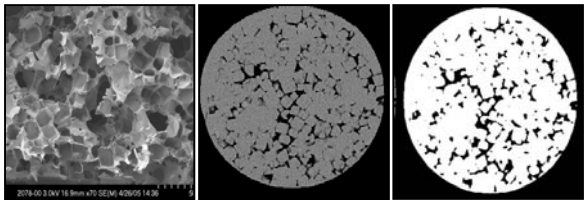

Figure 1: a) SEM of hydrogel cross-section, b) Micro- MR section and c) pore-solid delineation of OPF hydrogels with 75% salt porogen concentration of 100μm diameter salt particles.

The total number of cells cultured on the porous hydrogels was quantified with a DNA assay. Cells did not show significant proliferation over a time period of 7 days and similar cell numbers were observed on the scaffolds at different time points. Alkaline phosphatase (ALP) activity, an indicator of the osteoprogenitor cell's commitment to the osteoblastic phenotype, was measured and normalized by the total cell number for each sample. Although the difference in the ALP activity of the cells on hydrogel scaffolds with different porosities was not significant, the ALP activity of the cell-hydrogel composites was significantly higher than that of cells on tissue culture polystyrene. This indicates that the porous hydrogel scaffolds supported differentiation of the MSCs to the osteoblastic phenotype.

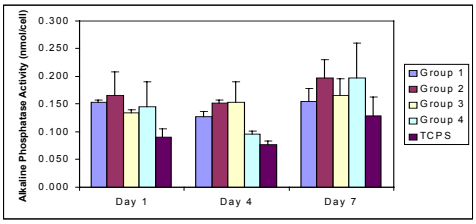

Figure 2: ALP activity of the MSCs on porous scaffolds.

Conclusions: Our results showed that micro MRI is an appropriate method for characterization of hydrogel porosity and interconnectivity. Moreover, porous hydrogels appeared to support the differentiation of MSCs and may find application in bone tissue engineering.

References: 1-Jo. S et al. Macromolecules 2001;34:2839-2844.
2-Maniatopoulos, C. et al. Cell Tissue Res. (1988);56:283-294

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